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Review

# Innate-like T cells in liver disease

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**Mammalian innate-like T cells (ILTCs), including mucosal-associated invariant T (MAIT), natural killer T (NKT), and γδ T cells, are abundant tissue-resident lymphocytes that have recently emerged as orchestrators of hepatic inflammation, tissue repair, and immune homeostasis. This review explores the involvement of different ILTC subsets in liver diseases. We explore the mechanisms underlying the pro- and anti-inflammatory effector functions of ILTCs in a context-dependent manner. We highlight latest findings regarding the dynamic interplay between ILTC functional subsets and other immune and parenchymal cells which may inform candidate immunomodulatory strategies to achieve improved clinical outcomes in liver diseases. We present new insights into how distinct gene expression programs in hepatic ILTCs are induced, maintained, and reprogrammed in a context- and disease stage-dependent manner.**

## Highlights

Mammalian innate-like T cells (ILTCs), including natural killer T (NKT), mucosal-associated invariant T (MAIT), and γδ T cells are tissue-resident unconventional T cells that share properties of innate and adaptive immunity.

ILTC subsets can secrete cytotoxic effector molecules and cytokines to clear out abnormal hepatocytes, initiate effector cell recruitment and inflammation, and contribute to regulating homeostasis and tissue regeneration in liver diseases.

ILTCs can contribute to both protective and pathogenic functions depending on the liver disease stage and tissue context.

ILTCs can contribute to liver inflammation and injury in different pathological conditions including viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), cirrhosis, and others.

Targeting ILTCs or modulating their functions may represent potential therapeutic strategies to help to treat liver diseases by aiming to restore immune balance and mitigating liver inflammation.

## Significance

As abundant tissue-resident lymphocytes, ILTCs play important roles in ensuring liver tissue integrity during homeostasis, infection, and non-infectious chronic perturbations. Pathological conditions, including viral infections, metabolic dysfunction, alcohol consumption, and others, can significantly influence the phenotype and function of ILTCs, further underscoring their dynamic role in liver immune responses across different disease contexts. Understanding the intricate interplay between ILTCs, other cells, and niches in liver diseases is essential for developing targeted therapeutic interventions to restore immune balance and mitigate pathology.

## ILTCs as emerging targets to modulate hepatic immunity

Liver diseases impose a substantial global health challenge because they lead to ~2 million human deaths annually, primarily driven by cirrhosis, viral hepatitis, and hepatocellular carcinoma (HCC) [1]. These pathologies intricately involve innate and adaptive immunity in which elevated systemic inflammation and immune dysfunction are the primary triggers of hepatic tissue injury [2]. Mammalian ILTCs including **natural killer T (NKT) cells**, MAIT, and γδ T cells represent abundant lymphocyte populations that share properties with both conventional αβ T cells and innate immune cells such as helper innate lymphoid cells (ILCs) and natural killer (NK) cells [3]. Recently, it has been recognized that ILTCs and their respective subsets can play both pathological and protective roles in liver disease [4]. Thus, modulating ILTC activity emerges as a promising therapeutic strategy for restoring immune equilibrium and facilitating liver tissue regeneration [5]. We summarize our current understanding of the role of ILTCs in the immunopathology of liver diseases. We highlight recent findings regarding the importance of ILTC functional subsets in liver pathologies and how distinct gene expression programs in hepatic ILTCs are induced, maintained, and reprogrammed in a context- and disease-stage-dependent manner. Novel insights into the potential of modulating ILTCs in liver disease using T cell receptor (TCR) ligands are also briefly discussed.

## The family of ILTCs

The group of mammalian ILTCs includes three major subsets: NKT cells, MAIT cells, and γδ T cells (see Figure I in Box 1). Unlike conventional peptide-reactive αβ T cells, which primarily recognize antigens presented by major histocompatibility complex (MHC) class I and class II molecules, ILTCs recognize a diverse range of self and non-self molecules through their semi-invariant [MAIT and **invariant natural killer (iNKT) cells**] or diverse (γδ T cells) TCRs [3] (Box 1). The evolutionary conservation of the MHC class I-like molecules CD1d and MR1 (that present antigens to NKT cells and MAITs, respectively) and the corresponding TCR specificities of MAIT and NKT cells [6–10] suggests that ILTCs serve a crucial role in host physiology by mediating non-self recognition and maintaining tissue homeostasis by orchestrating inflammation and tissue repair [11]. Despite their constrained TCR diversity, some ILTC subsets such as γδ T cell subsets exhibit a



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broader spectrum of antigen specificities [12]. Beyond TCR-dependent activation, hepatic ILTCs can be activated in a TCR-independent manner, a feature shared with innate immunity [13]. They can rapidly sense environmental changes through the expression of integrins, chemokines, and cytokine receptors, allowing them to promptly mount potent effector responses. Furthermore, both human and murine ILTCs share features of innate immune cells, such as their accumulation at barrier sites (gut mucosa, lung, and liver tissues) and an effector phenotype manifested before thymic egress (Box 2) [14]. Promyelocytic leukemia zinc finger (PLZF) has been identified as a shared master transcription factor for ILTC development, but with differences in expression between lineages [14,15]. Recent technological advancements have shed light on the shared and distinct transcriptomic profiles of ILTC subsets and have highlighted the importance of tissue context for ILTC function. For instance, using bulk transcriptomics and single-cell RNA sequencing (scRNA-seq), MAIT and NKT cells in mice and humans have been shown to harbor common transcriptomic profiles according to the tissue these cells reside in [10,15]. In humans, a comparison between circulating MAITs and hepatic MAIT cells demonstrated that phenotypic and functional diversity is predominantly determined by tissue localization, and also governs their responsiveness *ex vivo* [16]. The distinct transcription factor and effector molecule signatures of NKT, MAIT, and γδ T cells, as well as their respective functional subsets, are outlined in Figure I in Box 1.

## Pro- versus anti-inflammatory ILTC properties in liver pathogenesis

Human and murine hepatic tissue-resident ILTCs are characterized by their abundance, long-term maintenance, and capacity for self-renewal [3]. Patrolling within the liver sinusoids, ILTCs sense cytokines, **alarmins**, and **stress ligands** through cognate receptors, thus enabling swift secretion of either proinflammatory or tissue-protective factors [3,17]. However, the functional properties of ILTCs in the liver microenvironment are context-dependent and are controlled

### Box 1. Major ILTC subsets and their characteristics

Invariant natural killer T (iNKT) cells, or type 1 NKT cells, are characterized by the expression of semi-invariant αβ TCRs (Figure I). These allow recognition of antigens presented by the MHC class I-like molecule CD1d, notably self or foreign α-linked glycolipids [103]. By analogy to helper $CD4^+$ T cells, distinct iNKT cell subsets are characterized by hallmark type 1, type 2, and type 3 transcription factors and effector molecules. In laboratory mice, iNKT cells show lower frequencies in peripheral blood (0.2% of the T cell population) but are notably enriched in the liver where they constituting up to 30–50% of hepatic T cells [14,104]. In humans, NKT cells are less abundant, ranging from 0.05 to 1% of liver lymphocytes [105]. Type 2 NKT cells constitute another CD1-restricted subset that is characterized by more diverse αβ TCR repertoires and reactivity to various self or microbial lipid antigens, including sulfatide [106]. In naive mice, type 2 NKT cells are less abundant than the iNKT subset [107]. Type 2 NKT cells in human peripheral blood represent less than 1% but constitute ~6% of total $CD3^+$ cells [108].

MAIT cells are identified by the expression of invariant TCR α-chain polypeptides (Figure I) and a limited array of TCR β-chains [109,110]. They are restricted by the non-polymorphic MHC class I-related (MR1) molecule and recognize microbial agonist ligands derived from vitamin B2 (riboflavin) or vitamin B9 (folate) synthesis pathways [7,8]. Of note, there is a subset of non-MAIT MR1-restricted T cells with diverse TCRs that do not recognize vitamin B metabolites, and instead recognize tumor antigens that remain to be defined [111]. MAIT cells are more abundant in human peripheral blood where they comprise 1–10% of total $CD3^+$ T cells [112,113] compared to ~0.1% of $CD3^+$ T cells in mice [114]. Within the human liver, MAIT cells constitute up to 40% of total liver T cells [115], whereas in laboratory mice they only account for ~0.6% of total liver $CD3^+$ T cells [114].

γδ T cells express a γδ TCR composed of Vδ and Vγ chains, and diversify the TCR repertoire through V(D)J DNA recombination. Unlike αβ TCR, γδ TCRs often exhibit independence from MHC-mediated antigen presentation and recognition; the diversity of antigens remains less well defined but has been demonstrated for phospho-antigens and stress-induced ligands [116–118] as well as tumor-associated antigens [119]. Similarly to NKT and MAIT cells, there are considerable differences in γδ T cells between species. For example, γδ T cells account for 0.5–5% of human peripheral blood T cells but primarily inhabit epithelial barrier tissues such as the intestine (~40% of intestinal intraepithelial lymphocytes), skin (~70% of skin dermal T cells), and liver (6.6%) [120–122]. There are two major subsets in human that have preferential tissue tropism: $Vδ2^+$ cells, that are predominantly circulating and are abundant in lymphoid organs, and $Vδ1^+$ cells that are enriched in mucosal tissues. In human peripheral blood mononuclear cells, $Vγ9Vδ2^+$ T cells represent the most abundant γδ T cell population but these cells are not found in mice.

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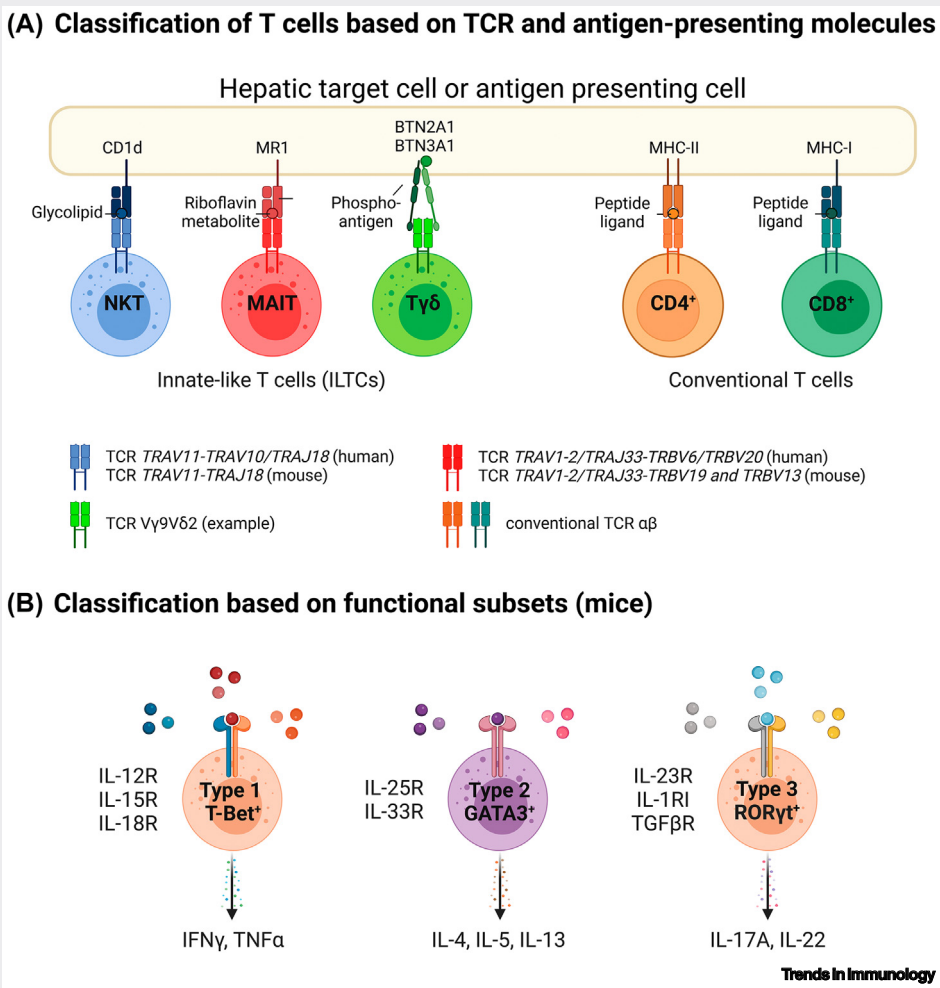


Figure I. Overview of innate-like T cell subsets. (A) Antigen-presenting cells (APCs) in the mammalian liver express MHC, CD1d, and MHC class I-related (MR1) proteins [146]. Conventional αβ $CD4^+$/$CD8^+$ T cells express highly variable TCRs that are responsive to peptide ligands presented by MHC class II or MHC class I, respectively. MAITs and NKT cells possess a more limited TCR repertoire [3,12]. Antigen presentation to γδ T cells is more diverse because some subsets of γδ T show adaptive features, but the antigens recognized by such γδ T cells are unknown [147]. Thus, the figure simplifies this process and only illustrates a single known mechanism for activating human $Vγ9Vδ2^+$ T cells that involves phospho-antigens and members of the butyrophilin (BTN) family such as BTN3A1 and BTN2A1 [116]. (B) Functional subsets (types 1, 2, and 3) are driven by key transcription factors, cytokines, and cytokine receptors. Type 1 subsets express T-box expressed in T cells (T-bet) and secrete interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α), while also expressing IL-12R, IL-15R, and IL-18R [148]. GATA3 expression distinguishes type 2 subsets, which are characterized by the secretion of IL-4, IL-5, and IL-13, as well as by IL-25R and IL-33R expression [149]. Type 3 phenotypes rely on retinoic acid receptor-related orphan receptor γt (RORγt), produce IL-17 and IL-22 [150], and express IL-23R, IL-1RI, and TGFβR. Abbreviations: IL-12R, IL-12 receptor; IL-1RI, interleukin 1 receptor type I; MHC, major histocompatibility complex; TCR, T cell receptor; TGFβR, transforming growth factor β receptor. Figure created with Biorender.

by shared adaptation mechanisms [18]. Indeed, ILTCs harbor various effector functions ranging from proinflammatory cytokine-mediated to direct hepatocyte killing; however, their activities in tissue repair and wound healing [19,20] can also promote fibrosis and cirrhosis. In this section we highlight shared pathways whereby ILTCs may act as a multilayered cellular network with partially overlapping functions, presumably adapting across different liver pathologies (Figure 1, Key figure). It should be noted that, despite their similarities, there are important inter-species

## Glossary

**Alarmins:** endogenous molecules released by damaged or stressed cells; they trigger an immune response by signaling the presence of tissue injury or infection.
**Alcoholic liver disease (ALD):** liver pathogenesis caused by chronic excessive alcohol consumption leading to steatosis, hepatitis, and cirrhosis.
**Autoimmune hepatitis (AIH):** a chronic liver disease characterized by immune-mediated destruction of hepatocytes involving autoantibodies and lymphocytic infiltration that cause inflammation.
**Autoimmune liver diseases (AILDs):** conditions in which the immune system mistakenly targets liver cells, causing chronic inflammation, liver damage, and impaired liver function.
**Cholangiocytes:** epithelial cells that line the bile ducts and are responsible for bile secretion and modification.
**γδ1 T cells:** predominantly reside in epithelial tissues and participate in immune responses against stressed or infected cells, particularly in mucosal and barrier tissues.
**Hepatic stellate cells (HSCs):** pericyte-like cells residing in the liver sinusoids that are crucial for liver homeostasis and fibrosis progression due to their activation into myofibroblast-like cells upon liver injury, leading to excessive extracellular matrix deposition.
**Invariant natural killer T (iNKT) cells:** a specialized subset of innate-like T cells characterized by their invariant TCR that recognizes glycolipid antigens presented by CD1d.
**Metabolic dysfunction-associated liver disease (MASLD):** conditions characterized by liver inflammation and damage due to metabolic factors such as obesity, type 2 diabetes, and dyslipidemia.
**Myeloid-derived suppressor cells (MDSCs):** a heterogeneous population of immature myeloid cells that suppress T cell activation and promote Treg activity, thus contributing to immune evasion and disease progression.
**Natural killer T (NKT) cells:** a diverse group of T cells that have features of both adaptive and innate immune cells. Type 1 NKT (NKT1) cells are characterized by IFN-γ production and play a role in enhancing antitumor and antimicrobial immune responses. NKT2 cells recognize diverse lipid antigens

Box 2. ILTC development

ILTCs arise from common lymphoid progenitors (CLPs) that migrate to the thymus for further development [14]. Studies in mice have shown that γδ T cells already develop and populate peripheral tissues at the fetal stage, thus preceding MAIT and NKT cell development. Promyelocytic leukemia zinc-finger protein (PLZF) is the master regulator governing ILTC development [123–125], and MAITs and NKT cells acquire effector phenotypes before thymic egress [126]. Thus, NKT1 (MAIT1, γδT1, T-bet$^+$), NKT2 (GATA3$^+$), and NKT17 (MAIT17, γδT17, RORγt$^+$) subsets can be already found in the thymus [127]. Whether or not there is cellular plasticity exists in different ILTC subsets remains unknown. Ligand presentation by CD1d and MR1 on thymocytes is necessary for NKT and MAIT cell development: *Mr1*$^{-/-}$ and *Cd1d*$^{-/-}$ mice lack MAIT cells and NKT cells, respectively. Early-life exposure to microbiota controls the thymic development of ILTCs [11]; indeed, MAIT cells in peripheral tissues are basically absent in germfree mice or mice colonized only by bacteria lacking riboflavin synthesis [19,128]. Peripheral NKT cell numbers are also altered in germfree mice where iNKT cells accumulate in the lung and colonic lamina propria, and fewer iNKT cells are found in the spleen and liver [129,130]. Early-life microbial imprints can subsequently permanently shape phenotype and function of tissue-resident ILTCs later in life [19,131].

differences, such as the relative abundance of MAITs in human liver and the scarcity of their murine counterparts, whereas the opposite holds true for NKT cells. Keeping these differences in mind should be a premise when interpreting data derived from murine studies.

The analogy to conventional peptide-reactive CD4$^+$ **T helper cell** responses [21] can help us to classify distinct ILTC subsets that convey pro- or anti-inflammatory properties in liver pathologies such as bacterial and viral infections, **autoimmune liver diseases (AILDs)**, **alcoholic liver disease (ALD)**, and **metabolic dysfunction-associated steatotic liver disease (MASLD)** (see Figure I in Box 1). Although this approach provides a framework to categorize ILTC functional states, we stress again that there are important differences across species. For example, human MAIT cells acquire a mixed type 1/type 3 phenotype, whereas murine MAITs present with distinct type 1 or type 17 differentiation programs, as recently demonstrated by scRNA-seq [10,16]. This has important implications when interpreting findings derived from murine model systems, and key findings should be validated in humans.

## Type 1 signaling

ILTCs may cause hepatocyte damage, in part by producing diverse cytokines involved in recruiting and stimulating other immune cells and cell death mediators; this in turn potentially exacerbates existing liver damage [22]. ILTCs secrete canonical type 1 cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and GM-CSF; they also engage in CD40/CD40L interactions to stimulate neighboring innate immune cells, and directly release death signals to hepatocytes and **cholangiocytes** via the Fas/Fas ligand (Fas/FasL) pathway together with perforin and granzymes (GzB) [23,24]. Type 1 cytokines recruit conventional αβ T cells and other proinflammatory cells to the liver, thus enhancing liver damage, as demonstrated in a xenobiotic-induced [2-octynoic acid coupled to bovine serum albumin (2-OA-BSA) immunization] mouse model of **primary biliary cholangitis (PBC)** where increased IFN-γ production of NKT cells upon α-galactosylceramide (α-GalCer) stimulation led to increased CD8$^+$ T cell infiltration and bile duct damage [25]. However, IFN-γ also has protective roles in facilitating viral clearance (discussed below).

## Type 2 responses

ILTC-secreted type 2 cytokines have both pro- and anti-inflammatory functionalities. For instance, IL-4 produced by type I NKT cells upon stimulation can promote liver inflammation through neutrophil recruitment in mice [26]. Furthermore, ILTCs can also play a crucial role in mitigating liver pathology by preserving immune balance through type 2 signaling. For instance, transforming growth factor (TGF)-β, IL-4, and IL-10 pathways can lead to the so-called 'M2-like' (anti-inflammatory) macrophage polarization, as suggested *in vitro* for MAIT cells derived from MASLD patients, and in mouse models of fatty liver disease using a methionine/choline-

presented by CD1d, produce IL-4 and IL-13, and contribute to allergic responses and immunoregulation. NKT10 cells have an IL-10-dependent regulatory function and suppress antitumor immune responses and autoimmune reactions.

**Primary biliary cholangitis (PBC):** a chronic liver disease characterized by progressive destruction of the small bile ducts within the liver.

**Primary sclerosing cholangitis (PSC):** a chronic liver disease characterized by progressive inflammation, fibrosis, and strictures affecting the intrahepatic and/or extrahepatic bile ducts.

**Regulatory T cells (Tregs):** a subset of CD4$^+$ T cells that maintain immune tolerance and prevent autoimmune responses by suppressing excessive immune activation.

**Stress ligands:** molecules expressed on the surface of cells in response to stress or damage; they can activate immune cells to eliminate the affected cells.

**T cell exhaustion programs:** a state where T cells become dysfunctional during chronic infections and cancer; T cell exhaustion is characterized by reduced cytokine secretion and effector function, as well as by enhanced expression of inhibitory receptors.

**T helper cells:** type 1 ($T_H1$) cells are a subset of CD4$^+$T cells that produce IFN-γ, IL-2, and TNF-β which activate macrophages and neutrophils to drive cell-mediated immunity and phagocyte-dependent responses. Type 3 T helper 17 ($T_H17$) cells are a subset of CD4$^+$ T cells that produce IL-17 and IL-22; they are involved in immune responses against extracellular bacteria and fungi, and contribute to autoimmune inflammation.

**Vδ2 T cell subsets:** distinct groups of γδ T cells that express the Vδ2 TCR chain which specifically recognizes phosphoantigens; γδ T cells play a significant role in immune responses against infections and cancers.

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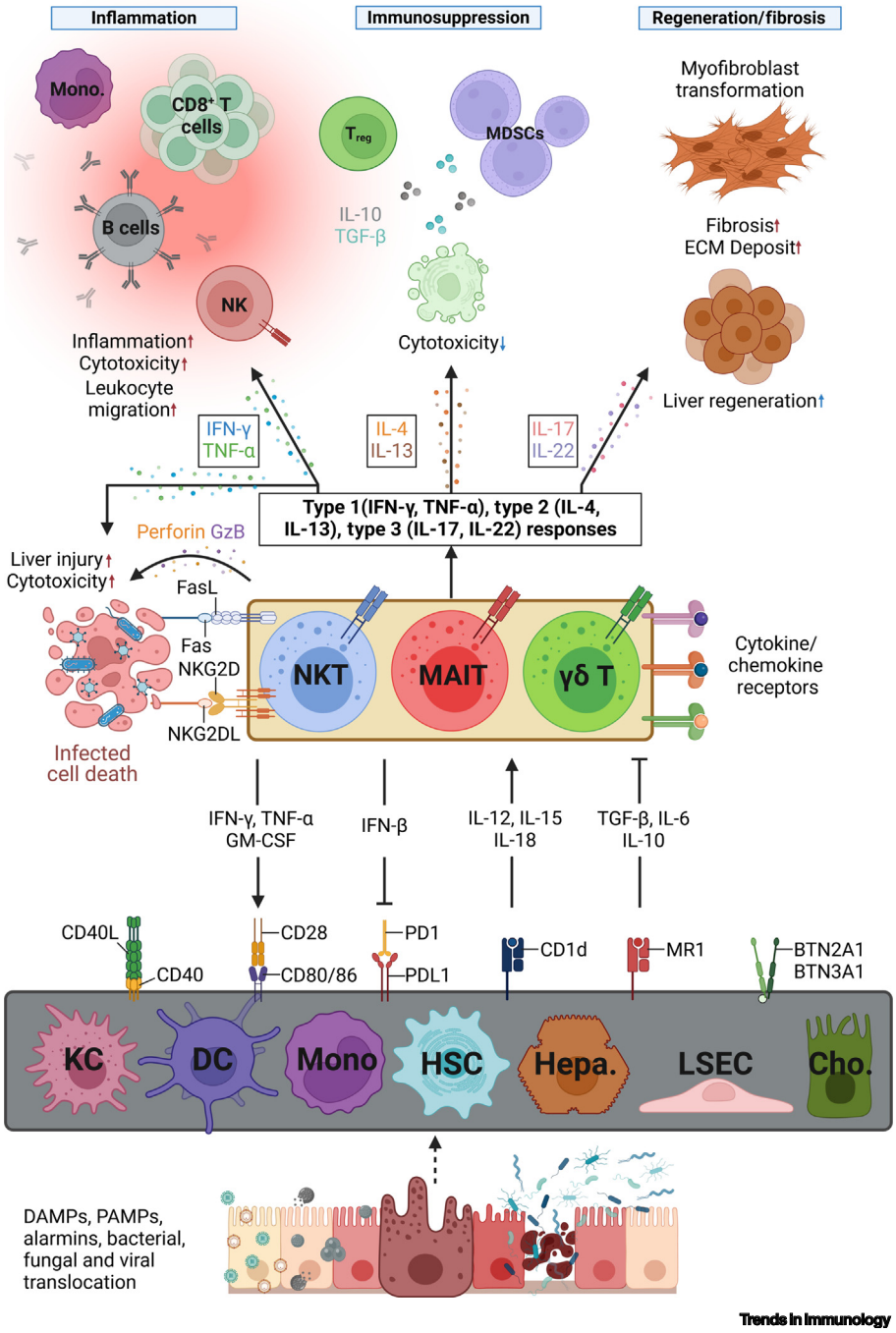
## Key figure

### Concurrent and overlapping mechanisms of innate-like T cells within the liver microenvironment

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deficient (MCD) diet [27]. Further protective roles of ILTC type 2 signaling were suggested from experiments in mouse models of sterile liver injury showing that iNKT cell-secreted IL-4 is associated with hepatocyte proliferation and wound healing as well as in a switch in hepatic monocyte phenotype (from Ly6C$^{high}$ to Ly6C$^{low}$).

### ILTCs and type 3 immunity

ILTCs, as major IL-17 cytokine producers in the human and mouse liver, are pivotal in modulating cellular responses within injured hepatic tissue. For instance, IL-17 accelerates MAFLD progression via neutrophil recruitment and by inducing reactive oxygen species (ROS) in wild-type (WT) (C57BL/6) versus *Il17ra*$^{-/-}$ mice fed a high-fat diet (HFD) [28]. Moreover, ILTC-associated IL-17A exacerbated fibrosis progression by activating **hepatic stellate cells (HSCs)**/hepatic myofibroblasts (HMFs), thereby promoting liver fibrogenesis through the expression of collagen and other extracellular matrix proteins; this was demonstrated in patients with AILD [29], ALD [30], and in mouse models of hepatotoxin carbon tetrachloride ($CCl_4$)-induced liver fibrosis [31,32].

Taken together, we posit that these pleiotropic pro- versus anti-inflammatory ILTC roles are context-dependent. Thus, in the liver, the balance or imbalance between these conserved signals (types 1, 2, and 3) can largely influence the actions of ILTCs in health and disease.

## Hepatitis B and C viruses (HBV and HCV)

HBV and HCV are hepatotropic viruses that can cause acute and chronic hepatitis, and are major risk factors for liver cirrhosis and HCC development in humans [33,34]. The precise roles of ILTCs in either orchestrating viral clearance or fostering virus persistence within the liver remain insufficiently understood.

Because of their ability to recognize virus-infected cells directly through NK cell-related receptors {such as natural killer group 2 member D (NKG2D) [35]} and indirectly through innate cytokine receptors and type I IFN receptors, ILTCs might represent the first line of defense against hepatotropic viruses; indeed, ILTCs cells can respond to infection within hours to a few days [12,36,37]. Of note, many studies investigating the role of ILTCs from patient samples have lacked important controls such as adequate comparisons between ILTCs and conventional αβ tissue-resident memory T cells (TRMs). These limitations make it difficult to determine whether some ILTC features are also shared with conventional T cells. Nevertheless, the evidence suggests that ILTCs could contribute to controlling HBV and HCV infections through IFN-γ secretion which correlates positively with infection outcomes [38–40]. For example, in HBV transgenic mice

Figure 1. Bacterial translocation, viral infection, and hepatocyte damage release danger-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP) molecules and alarmins that activate antigen-presenting cells (APCs) [146]. Within the liver microenvironment, APCs present antigens (see Figure I in Box 1) [7,40,116] and costimulatory molecules to innate-like T cells (ILTCs) [29,40,97,145]. APCs also secrete cytokines/chemokines to modulate mammalian ILTC function [146]. In response, ILTCs produce effector molecules to expand or restrain APCs (e.g., via IFN-β). Upon activation, ILTCs may also directly kill via Fas–FasL or NKG2D–NKG2DL interactions, or through perforin and granzyme B (GzB) secretion [23,24,99,146,151]. Furthermore, ILTCs adopt effector phenotypes (types 1, 2, and 3) to influence the liver microenvironment (Box 1). Type 1 cytokines drive inflammation by initiating effector cell migration such as for NK, B, CD8$^+$ T cells, and monocytes [23,135]. Conversely, Type 2 cytokines such as IL-4 and IL-10 suppress immune-related cytotoxicity, via **regulatory T cells (Tregs)** or myeloid-derived suppressor cell (MDSC) activation [152–155]. Type 3 cytokines (e.g., IL-17 and IL-22) are involved in hepatocyte injury and stimulate hepatic stellate cells (HSCs), thus contributing to liver extracellular matrix (ECM) deposition and fibrosis [156,157]. Abbreviations: BTN2A1/BTN3A1, butyrophilin subfamily 2/3 member A1; Cho, cholangiocyte; DC, dendritic cell; MAIT, mucosal-associated invariant T cell; Mono, monocyte; Hepa, hepatocyte; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; MR1, major histocompatibility complex, class I-related protein; NK, natural killer; PD-1/PD-L1, programmed cell death protein 1/ligand 1. Figure created with Biorender.

[41], activation of NKT cells with α-GalCer led to increased production of IFN-γ and IFN-α/β, resulting in reduced HBV replication [39].

Although the abovementioned studies suggest that similar mechanisms operate across different ILTC populations (e.g., through IFN-γ secretion), distinct features have also been demonstrated in MAIT cells from acutely infected HCV patients – these cells display dysfunctional effector function upon *in vitro* stimulation with *Escherichia coli* lysate or with IL-12/IL-18, as evidenced by flow cytometric cytokine measurements [42]. MAIT cell effector function was not restored after viral clearance, but γδ T cells showed an active phenotype by flow cytometry in this patient cohort, suggesting possible distinct roles between these ILTC subsets, but this is not yet fully understood [42].

These studies highlight the necessity for high-dimensional technologies such as spectral cytometry, cytometry by time of flight (CyTOF), or scRNA-seq for comprehensive ILTC subpopulation analysis. This will be important because ILTC subpopulations may exhibit distinct roles during viral hepatitis, and a better understanding of these subpopulations will be essential to ultimately target ILTCs for therapeutic purposes [43]. Examples of these functions have been reported for human γδ T cells where different hepatic **Vδ2 T cell subsets** from HBV-infected patients suppressed the production of αβ type 17 T helper ($T_H17$) cells *in vitro*, suggesting that the former might be involved in preventing HBV-induced liver damage through this mechanism [44]. Conversely, in chronic HBV infection, other immunomodulatory functions of ILTCs might apply. This has been suggested for mouse IL-17-producing Vγ4 T cell subsets which recruit **myeloid-derived suppressor cells (MDSCs)** that suppress αβ T cell function and in turn facilitate HBV infection [45]. These studies highlight the level of granularity that is needed for detailed exploration of complex ILTC interactions in different compartments (e.g., circulation vs. tissue); indeed, ILTC subpopulations show plasticity and adapt to dynamic environmental alterations that differ in acute versus chronic liver disease [43].

In chronic HBV and HCV patients, it is well established that circulating ILTC numbers are reduced and this correlates with the disease stage; for example, the degree of fibrosis/cirrhosis [38,46–51]. However, whether this decline reflects tissue recruitment or increased cell death of these cells remains unclear. Furthermore, intrahepatic MAIT, NKT, and γδ T cell frequencies are diminished in chronic HBV and HCV patient populations compared to healthy controls, suggesting that they are potentially sequestered in other tissues [38,46–48,50,51]. Phenotypically, ILTC surface marker expression changes resemble **T cell exhaustion programs** of conventional $CD8^+$ T cells during chronic HBV and HCV infections [52] that are marked by increased expression of receptors such as programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte-associated protein 4 (CTLA-4), T cell immunoglobulin and mucin-domain containing 3 (TIM3), CD38, and CD39 compared to healthy individuals [46,50,53–55]. Whether the underlying mechanisms such as chronic TCR stimulation resemble those governing conventional αβ T cells warrants further exploration. We propose that precise investigation of putative ILTC exhaustion programs and comparison with conventional αβ T cell counterparts may be necessary to better understand the functions of these cells. Of note, altered ILTC properties such as the acquisition of type 2 polarization during chronic HBV or HCV infection might hasten liver fibrosis and HCC [56–59]. Indeed, murine iNKT cells have been associated with HSC activation through IL-4 and IL-13 secretion [59]. This could potentially offer therapeutic options to target ILTCs depending on the disease context (Box 3).

The studies mentioned above highlight the presumed protective roles of ILTCs in hepatotropic viral infection, including virus clearance. Nonetheless, during chronic inflammation and viral persistence, ILTCs may undergo functional alterations that could also promote fibrosis and cirrhosis (discussed below).

Box 3. Targeting ILTCs to modulate liver disease

From a therapeutic perspective, there are several approaches to targeting ILTCs in liver disease. Using ILTC TCR ligands as antiviral therapies seems promising in overcoming ILTC exhaustion and polarizing the cells towards antiviral $T_H1$/NK-like functional states. The prototypic iNKT cell TCR ligand, glycosphingolipid α-galactosyl ceramide (α-GalCer), has been investigated in Phase 1/2 studies as a monotherapy for chronic HBV [132] and chronic HCV patients [133] where it showed moderate immunomodulatory effects. Preclinical studies suggest that conjugating α-GalCer with an HBV core antigen fragment $HBcAg_{93–100}$ can enhance vaccine efficacy in HBV transgenic C57BL/6 mice [134].

Furthermore, activating TCR ligands may polarize ILTCs cells towards a $T_H1$/NK-like phenotype with antitumor properties. Moreover, MAIT cell expansion through systemic application of the MAIT ligand 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and the TLR9 ligand CpG enhances MAIT-dependent antitumor immunity in murine liver metastasis models [135]. Similarly, the glycolipid ligand αGalCer shows antitumor efficacy in several murine tumor models [136,137], although iNKT cell anergy upon repetitive α-GalCer stimulation has dampened enthusiasm for this approach [138,139]. Using TCR ligands for stimulation of γδ T has focused on polyclonal Vγ9Vδ2 T cells that can be activated through amino bisphosphonates (e.g., zoledronate or pamidronate) in combination with IL-2 [140].

In the context of fibrosis, blocking MAIT cell activation via the inhibitory MR1 ligand acetyl-6-formylpterin (acetyl-6-FP) was found to induce fibrosis regression in mice [98] and might offer further therapeutic potential. Alternative experimental strategies to activate the antiviral or antitumoral properties of hepatic ILTCs have been described, including supplementation with IFN-α, IL-2, IL-12, IL-15, IL-18, thymosin-α1, or β-glycosphingolipids, as well as CD28/CD80 activation and chemoattractant CXCL16 and PD-1/PD-L1 blockade [141–145].

## Autoimmune hepatitis and chronic cholestatic liver diseases

**Autoimmune hepatitis (AIH)**, **primary sclerosing cholangitis (PSC)**, and primary biliary cholangitis (PBC) are chronic hepatobiliary disorders that are characterized by immune-mediated destruction of hepatocytes or biliary epithelial cells [60]. Although the mechanisms underlying AILDs are incompletely understood, they emerge in genetically predisposed individuals and are triggered by environmental factors such as viral and metabolic disturbances. Moreover, inadequate regulatory immune control of hepatic inflammation may eventually cause the breakdown of immune tolerance, resulting in chronic liver injury [61].

ILTCs exhibit multiple functions in AILDs. On the one hand, their ability to stimulate HSCs, direct hepatocyte cytotoxicity, and recruit other immune cells potentially contributes to hepatic immunopathology and tissue injury [62]. This is exemplified by type 1 iNKT cells which have been reported to exert pathogenic effects, such as through IFN-α, as demonstrated in mouse models of concanavalin A (ConA)-induced AIH [63], as well as though IFN-γ, as shown in mouse models of PBC induced by 2-OA-BSA immunization [25]. Furthermore, MAIT accumulation in the portal venous system of AIH patient liver samples suggested their potential contribution to tissue injury by producing granzyme B *ex vivo* [64] – a phenomenon that was observed in MAIT cells from PBC patients [65] as well as in **γδ1 T cells** [66]. On the other hand, the immunomodulatory and tissue repair capabilities of ILTCs might help to counter disease progression. For instance, a recent study showed that genetic ablation of *Gpabar1* exacerbated hepatitis severity by promoting a proinflammatory NKT1 phenotype, whereas GPBAR1 agonism alleviated liver damage. In this case, induction of a regulatory NKT10 cell subset and expansion of IL-10-secreting type 2 NKT cells were implicated in protecting against acute liver injury in ConA- and α-GalCer-induced AIH mice [67]. From another angle, ILTC type 17 responses, mediated through IL-17/IL-22 signaling, may be important in enabling AILD pathology. ILTCs derived from AILD patients induce an activated, proinflammatory, and profibrogenic phenotype in human HSCs *in vitro*, partially through IL-17 production, which might contribute to HSC-mediated liver fibrosis [29].

Crosstalk between ILTC subsets is emerging as a concept; for instance, lamina propria MAIT cell-derived IL-17A has been reported to drive iNKT cell overactivation, and the MAIT–IL-17A–iNKT axis has been proposed as a putative therapeutic target in ConA-induced hepatitis in mice [68]. Although ILTC subsets present shared mechanisms, examples of their distinct features and

crosstalk with other ILTC subsets underscore the intricate nature of liver immunity mediate by these cells. For instance, γδ17 T cells demonstrate potential protective roles in AIH, where Vγ4 γδ T cells negatively regulate NKT cell function through IL-17A-dependent mechanisms in ConA-induced hepatitis in mice [69]. Conversely, in Jα18 knockout BALB/c mice (*Jα281*$^{-/-}$) with AIH, γδ T cell-derived IL-17A was implicated in mediating hepatocyte damage [70]. These findings suggest a pathogenic role for iNKT cells and a protective role for Vγ4 γδ cells. However, thorough examination of ILTCs and their subsets across disease models and stages is warranted to better contextualize these findings.

Aside from their interplay with other immune cells, ILTCs engage in constant exchange with parenchymal cells including cholangiocytes. These parenchymal cells function as antigen-presenting cells that present lipid antigens via CD1d to iNKT cells in cholangiopathies [71,72] and microbe-derived MAIT cell ligands through MR1 in *in vitro* stimulation assays [73]. Recent work uncovered the existence of endogenous MAIT ligands, such as a sulfated bile acid (cholic acid 7-sulfate, CA7S) which binds to MR1 and is recognized by human MAITs, that induce a homeostatic gene signature with *CXCR4*, *KLF2*, *TCF7*, and *IL7R* upregulation [74]. Nonetheless, the impact of microbe-derived ligands and endogenous ligands on ILTC phenotype and function remains largely unexplored. Of note, neuroimmune interactions have also been implicated in the regulation of hepatic iNKT cell function in murine models of stroke [75]; specifically, blockade of noradrenergic innervation following stroke was protective to WT mice but not in iNKT cell-deficient mice. Selective immunomodulation of iNKT cells with the specific activator α-GalCer resulted in proinflammatory cytokine production and prevented infections following stroke. These observations offer further insight into the complex role of NKT cells in chronic liver immunopathology.

## Metabolic dysfunction and alcohol-associated liver diseases

MASLD and ALD comprise a spectrum of hepatic disorders marked by abnormal fat accumulation that is triggered by dysregulated metabolic processes and alcohol intake [76]. Both ALD and MASLD encompass various impairments including hepatocyte apoptosis, hepatic inflammatory responses and fibrotic changes, and gut dysbiosis [77], leading to cirrhosis, liver failure, and HCC development [76]. Chronic alcohol consumption corresponds to the abnormal development, activation, and maturation of ILTCs in ALD [30,32,78,79]. The complex functionalities of ILTCs and their respective subsets have been demonstrated in numerous contexts. For instance, ALD can be mitigated by inhibiting iNKT cells through retinoids or by activating type 2 NKTs with sulfatide in alcohol-consuming C57BL/6 mice, as evidenced by reduced serum alanine transaminase (ALT) levels and decreased inflammation in liver sections upon hematoxylin/eosin (H&E) staining [80]. Mechanistically, chronic ethanol consumption elevates CD1d expression in enterocytes, leading to the activation iNKT cells in mesenteric lymph nodes that subsequently migrate to the liver and promote hepatocyte apoptosis in ethanol-fed intestinal epithelial cell-specific peroxisome proliferator-activated receptor γ-deficient mice (*Pparg*$^{\Delta IEC}$) [81]. In chronic and single-binge ethanol consumption mouse models, seemingly paradoxical results have been obtained because iNKT-deficient Jα18$^{-/-}$ (*Jα281*$^{-/-}$) mice showed enhanced histological hepatosteatosis with fewer NK cells and lower IFN-γ production (by flow cytometry) compared to controls [82]; the results suggested a possible protective role for IL-10-producing iNKT (NKT10) cells. Consequently, future research should focus on the characteristics of ILTCs at different stages of ALD and discern the intricate relationship between these cells and the immune network of the liver.

Numerous murine models of MASLD and MASH (metabolic-dysfunction associated steatohepatitis) have been developed that are induced through diet, toxins, or genetic engineering, and which

partially mimic human pathology [83]. Recent studies have suggested that ILTCs have protective roles in NAFLD progression, and altered hepatic ILTC frequencies were observed by flow cytometry in C57BL/6 mice fed a HFD or MCD [27,84]. Furthermore, NKT-deficient or MAIT-deficient *Cd1d*$^{-/-}$/*Mr1*$^{-/-}$ MASLD/MASH mice showed exacerbated hepatic fat accumulation, inflammation, and steatosis as assessed by histopathology, suggesting a protective role of NKT cells [27,85,86]. However, findings derived from these knockout mouse models remain to be carefully assessed, particularly in light of recent reports that, for example, CD1d is capable of reverse and intrinsic signaling which might also contribute to the phenotype of these mice (reviewed in [87]). Of note, scRNA-seq has revealed intricate relationships between ILTCs and the surrounding hepatic environment: in HFD mouse models, Kupffer cell-1 (KC-1) cells transitioned into cells with proinflammatory phenotypes and were reported to interact with iNKT cells via CD206-mediated crosstalk and drive hepatic homeostasis [88].

Although ILTCs have been suggested to have protective roles in MASLD, opposing functions have also been reported. This was exemplified by the induction of type 3 ILTC responses where hepatic ILTCs were found to be a significant source of IL-17 in both adipose tissue and the liver [89], and could potentially contribute to MASLD progression. γδ T cell deficiency and IL-17 neutralization also mitigated steatohepatitis and liver damage in HFD-fed mice. IL-17 was implicated in exacerbating MASLD progression by recruiting neutrophils, stimulating ROS production, activating HSCs and KCs, and also expanding the population of inflammatory conventional CD4$^{+}$ T cells [28,90,91]. Notably, an interplay between ILTCs and the gut microbiome has been suggested whereby *Akkermansia muciniphila* prevented the induction of Toll-like receptor 2 (TLR2)-activated γδT17 cells and proinflammatory M1-like macrophages in HFD-fed mice [92]. Therefore, the influence of the microbiome in hepatic ILTC functions is an area that merits further investigation.

## Fibrosis/cirrhosis

Liver fibrosis arises as a consequence of continued inflammatory damage to hepatic tissues, resulting in excessive collagen accumulation and architectural changes within the liver parenchyma via HSCs [93].

ILTCs can either promote or diminish cirrhosis development through shared molecular pathways. For instance, NKT cell-mediated type 2 responses, that are characterized by IL-4 and IL-13 secretion, have been associated with HSC proliferation and activation based on *in vitro* studies using peripheral blood mononuclear cells (PBMCs) from patients with HBV-related cirrhosis [94]. Similarly, in HBV transgenic mice treated with $CCl_4$ [59], NKT cell recruitment to the injured liver occurred through C-X-C chemokine receptor type 6/chemokine ligand 16 (CXCR6/CXCL16) signaling, as demonstrated by anti-CXCL16 antibody neutralization and two-photon microscopy imaging of *Cxcr6*$^{+/GFP}$ knock-in mice [95–97]. Type 3 polarized ILTCs are also increasingly being recognized as profibrogenic immune cell populations in patients with AILD [29], ALD [30], and $CCl_4$-induced fibrosis [31,32]. For instance, MAIT cells isolated from PBMCs from AILD patients were found to modulate HSCs *in vitro* and induce a profibrogenic phenotype via IL-17 secretion [29]. Furthermore, MAIT cell-deficient *Mr1*$^{-/-}$ mice were protected from liver fibrosis compared to WT C57BL/6 mice (as shown by Sirius red staining); targeting ILTCs might therefore have potential for fibrosis treatment and prevention [32]. Of note, inhibiting MAIT cells with acetyl-6-formylpterin (Ac-6-FP, Box 3) induced a phenotype shift of liver monocyte-derived macrophages as assessed by flow cytometry, emphasizing a connection between ILTCs and innate immune cells [98].

The role of ILTCs in fibrosis is a double-edged sword, highlighted by γδ T cell subsets in mice – their cytotoxicity against HSCs can have a protective role, whereas IL-17A expression may

have a detrimental role. On the one hand, CCR6$^+$ γδ T cells accumulate in fibrotic livers in close proximity to HSCs, as seen by immunohistochemistry, and restrict fibrosis via IL-17 and IL-22 production in murine $CCl_4$-mediated liver injury mice [99]. Mechanistically, CCR6$^+$ murine γδ T cells may regress liver fibrosis, as shown by increasing HSC apoptosis in coculture assays through Fas/FasL [99]. Moreover, IFN-γ-producing γδ T cells can eliminate activated HSCs in coculture by activating natural cytotoxicity-triggering receptor 1 (NKp46), TNF-related apoptosis-inducing ligand (TRAIL), and FasL mechanisms, suggesting that they might be protective against liver fibrosis [100]. Indirect mechanisms of actions have also been reported where NK cell-mediated cytotoxicity against HSCs *in vitro* was enhanced in WT compared to *Tcrd*$^{-/-}$ mice [100]. Thus, these studies highlight shared pathways in ILTCs that lead to fibrosis and cirrhosis; they also offer a glimpse into the distinct features of ILTC subsets which may be present at various disease stages.

## Concluding remarks

This review emphasizes the potential mechanisms and characteristics of ILTCs in diverse liver diseases. However, comprehensive, multidimensional investigation across space and time at the tissue level will be necessary to elucidate the precise mechanisms involved (see Outstanding questions). Beyond that, better model systems to study ILTC biology will be required, as has become evident because the widely used TCR *Jα18*$^{-/-}$ mouse strain that lacks iNKT cells also largely lacks MAIT cells [101]. Improved murine model systems [102] will be crucial to dissect the contribution of each cell ILTC subset to liver disease. Moving forward, we must expand our current understanding of ILTC cells in different disease contexts to fully capture their vast spectrum of effector functions and fully capitalize on their promising immunomodulatory functions in patients with liver disease and beyond.

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### Declaration of interests

The authors declare no competing interests.

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## Outstanding questions

Do the findings from murine models apply to humans, such as ILTC functions mediated through IL-17? Are there other cell types in humans that predominantly drive type 3 responses, such as conventional CD4$^+$ T cells, ILCs, or neutrophils? The major numerical and functional differences between ILTCs in model systems (mice) and humans require parallel analyses.

Are some of the non-redundant features of hepatic ILTC subsets due to the occupation of distinct microniches within the liver (e.g., intravascular vs. extravascular vs. intrasinusoidal)? It is increasingly appreciated that the tissue context of tissue-resident lymphocytes shapes their phenotype and function, but how this applies to ILTCs remains unknown.

How does the commensal microbiome shape hepatic ILTC communities through different disease stages? The reactivity of ILTCs to microbe-derived ligands suggests that such connections can drive ILTC reactivity, but this has not been studied in the context of liver disease.

How do neuroimmune interactions in different disease settings shape ILTC function at the systems level? This is another regulatory circuit that has gained attention in recent years, but compelling data on ILTCs in liver disease and neuroimmune interactions are lacking.

Are there further (endogenous) ligands for human ILTCs, especially for MAIT cells, type 2 NKT cells, and non-Vγ9Vδ2 γδ T cells? What mechanisms of antigen presentation and recognition apply? This is important when aiming to design ILTC TCR ligands as putative therapeutic strategies to modulate liver disease.

Can the application of activating or inhibitory ILTC TCR ligands modify liver diseases in a clinically meaningful way? Data from mouse models might not apply to human disease, and this needs to be systematically tested in clinical trials.

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